The effects of platelet-rich plasma(PRP) in combination with anorganic bovine bone(Bio-Oss®) on the early wound healing of rabbit cranial defects

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I. Introduction

Predictable regeneration of large alveolar defects with complex morphology can pose a significant clinical challenge, particularly when there is a significant vertical component involved. The presence of sufficient bone volume is an important prerequisite for dental implant placement[1,2,3,4]. Guided bone regeneration (GBR) is an accepted surgical procedure intended to increase the quantity and quality of host bone in localized defects of the alveolar ridge[5]. Methods described to increase the rate of bone formation and to augment the bone quantity include the utilization of autografts, allografts, xenografts, and alloplastic bone substitutes[6].

Autogenous bone is considered the "gold standard" for grafting alveolar defects. Despite being highly effective, these techniques subject patients to a second surgical site, which may increase morbidity, hospital stay, recovery, and cost[7]. There is also a greater risk for wound infection, more blood loss, and a slower return to normal function. It is also reasonably challenging to contour and can undergo significant and unpredictable resorption[8]. For this reason, researchers continue to work toward the development of a graft materal that is osteogenic, osteoinduction, and osteoconductive.

Allogenic bone is the most commonly used alternative to the autogenous harvest. The allografts most commonly used are demineralized freeze-

*This study was supported by a grant of the Korea Health 21 R & D Project, Ministry of Health & Welfare, Republic of Korea (03-PJ1-PG1-CH08-0001).

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deried allograft (DFDBA) and freeze-dried bone allograft (FDBA), and controversy exists with respect to the osteoinductive potential of these materials. It has been shown that the inductive capacity varies for DFDBA processed from different bone banks, and even different batches from the same bank respond differently. Also, the bioactivity of DFDBA seems to be dependent on the age of the donor, since the younger the donor, the more osteoinductive properties in the graft material[9]. Controversial results and patient's concerns about disease transmission have encouraged the development of xenografts and alternatives. Both have shown good biocompatibility and osteoconductive potential, but the clinical outcomes with these bone substitutes are unpredictable[10,11,12,13]. The lack of predictability in osseous regenerative procedures using bone grafts suggests that improvement in the osteoinductive and oeteoconductive properties of these materials is highly desirable.

Clinicians are constantly attempting to improve the results obtained with anorganic bovine bone alone by adding barrier membranes[14], fibrin glue[15,16], platelet concentrates[17], autogenous bone[18,19], osteogenic protein [19], and other nonautogenous grafting materials[20]. In 1990, Gibble and Ness[21] introduced fibrin glue, alternatively referred to as fibrin sealant or fibrin gel, a biomaterial that was developed in response to the necessity for improved hemostatic agents with adhesive properties. Platelet-rich plasma gel is an autogenous modification of fibrin glue that has been described and used in various applications with apparent clinical success[22]. PRP is potentially useful as an adjunct to allograft and xenograft materials in oral and maxillofacial bone and implant reconstructive surgery.

Platelets are very important in the wound healing process. They quickly arrive at the wound site and begin the coagulation process. They release multiple wound healing growth factors and cytokines, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1), vascular endothelial cell growth factor (VEGF), platelet-derived epidermal growth factor (PDEGF), platelet factor 4[23,24]. These growth factors are thought to contribute to initial bone regeneration and increase vascularity, and vital features of heal the bone graft[25,26,27].

The aim of the this study is to evaluate the effect of PRP on the early wound healing of rabbit cranial defects.

II. Materials and Methods

1. Animal surgical procedure

Fifteen New Zealand white male rabbits between 2.8 and 4 kg were included in this randomized, blinded, and prospective study. Each rabbit was anesthetized with Ketamine Hcl (5 mg/kg) and Xylazine Hcl (1.5 ml/kg). The fur was shaved over the cranium, which was prepared and draped in a sterile fashion. An incision was made to the bony cranium and the periosteum was reflected. By means of a trephine bur (external diameter : 8mm), four standardized 'through-and-through' bone defect were created with copious irrigation. The four cranial defects were randomly grafted with Bio-Oss® (Geistlich, Wolhusen/Switzerland) only, Bio-Oss® with PRP, PRP only, and no graft as a control(Figure 1). The four defects were covered with nonresorbable PTFE membrane (Tefgen®, Lifecore Biomedical, Inc, U.S.A.). The wound was closed with resorbable suture materials, and the rabbits were extubated and allowed to recover(Table 1). At the end of the surgical procedure, all animals received a single intramuscular injection of antibiotics Gentamicin (0.1 ml/kg).

Table 1. The four groups randomly grafted at the cranial defects.

Group	n	Graft materials	Membrane
control	5	no graft	PTFE (Tefgen®)
PRP	5	PRP	PTFE (Tefgen®)
Bio-Oss®	5	Bio-Oss®	PTFE (Tefgen®)
Bio-Oss® with PRP	5	Bio-Oss® with PRP	PTFE (Tefgen®)

2. PRP Preparation

The 10 mL of autogenous blood drawn from each rabbit was combined with 1.5 mL of Anticoagulant dextrose citrate (ACDC) to prevent coagulation. The blood was spun in a centrifuge (Placon, Oscotec, Korea) at 2,000G for 3 minutes to separate the plasma containing the platelets from the red blood cells. The plasma was drawn off the top of the test tube, and centrifuged for an additional 5 minutes at 5,000G to separate the platelets. The platelet-poor plasma (PPP) was separated from the PRP and the buffy coat. The buffy coat and the PRP, approximately 1 mL, were resuspended and added to the grafting material within minutes. One thousnad units of topical thrombin powder (Dirabine®, Korea United Pharm. inc, Korea) was reconstituted with 1 mL of 10% calcium gluconate (Calmia®, Korea United Pharm. inc, Korea)(Figure 2).

Platelet counts were performed on six samples, including a peripheral blood count, a PPP count, and a PRP count. The platelets were counted with a standard hemocytometer (Sigma, USA), and the total was calculated for each sample.

3. Evaluation

Rabbits were killed using phentobarbital, 100 mg/kg intravenously at 1,2, and 4 weeks. There were 5 rabbits in each group. The entire cranium was removed with a reciprocating saw, without encroaching on the grafted areas(Figure 3, A).

1) Radiographic evaluation

Radiographs were taken of the rabbit calvaria in its entirety before histologic sections were performed. A aluminum step-wedge was used in each radiograph for comparison(Figure 3, B). The radiographs were scanned and images were analyzed with a ImageJ 1.31v software on a IBM computer.

2) Histologic evaluation

The rabbit calvarias were fixed in 4% paraformaldehyde, and decalcified in hydrochloric acid decalcifying solution (Fisher Scientific, Tustin, CA) at 4℃ for 2-4 weeks. It was embedded in paraffin and cut into 6㎛ thickness. The sections were stained with H&E and observed by optical microscope.

3) Statistical methods

Numerical data was presented as mean plus one standard deviation. One way analysis of variance (ANOVA) with fisher's Tukey test was used for multiple comparisons to compare with the control. The probability level of $p < 0.05$ was regarded as statistically significant.

III. Results

1. Platelet count

Platelet counts that the PRP preparation technique used in this study produced a highly concentrated source of platelets. The mean peripheral blood platelet count was 150,500/㎣, with a range of

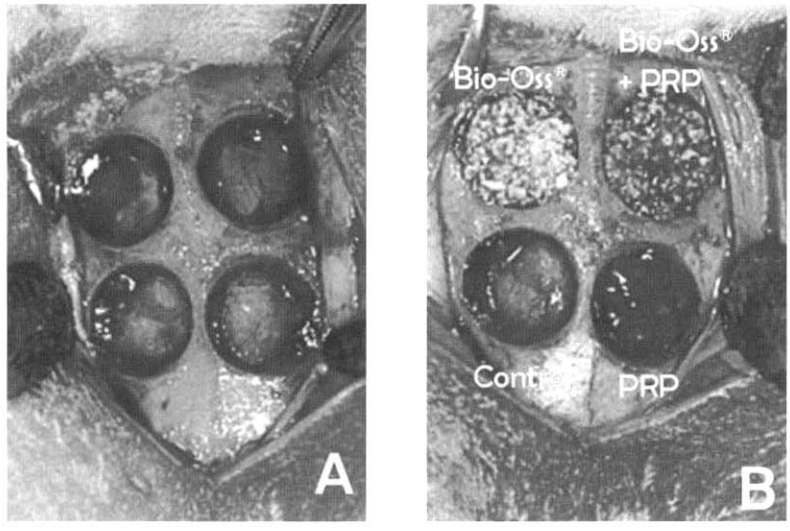

Figure 1. Photographs of the surgical sites
A, Rabbit cranium with surgical sites prepared
B, Rabbit cranium with surgical sites grafted

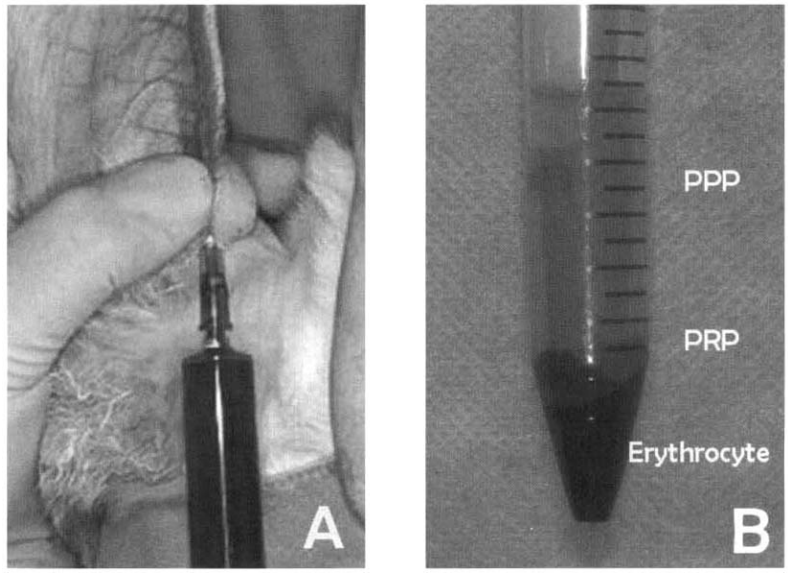

Figure 2. Preparation of platelet-rich plasma
A, Blood was taken from the marginal ear vein
B, Separation of three layers after second centrifuge

112,000 to 174,000/㎣. The mean platelet count PPP was 34,000/㎣, with a range of 10,000 to 60,000/㎣. The mean platelet count in PRP was 606,000/㎣, with a range from 425,000 to 752,000/㎣. These values confirmed the platelet sequestration ability of the process and quantified the concentration as 402% of baseline platelet counts(Table 2; Figure 4).

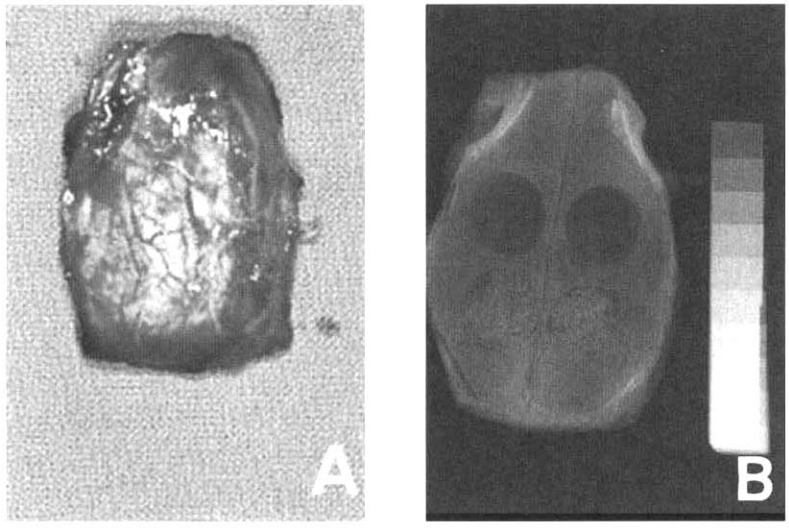

Figure 3. Gross and radiographic examination of surgical site
A, Rabbit cranium specimen was taken at 4 weeks
B, Radiograph of a rabbit cranium harvested after 4 weeks of healing

2. Radiographic evaluation

Figure 5 demonstrates the bone density as determined radiographically. Bio-Oss® group and Bio-Oss® with PRP group showed a significant increase in radiographic bone density when compared to the control and PRP group at all 3 time points($p<0.01$). However, at no time was there a statistically significant difference in radiographic density when Bio-Oss® alone was compared to Bio-Oss® with PRP group($p>0.05$). There is no significant difference in radiographic density when control was compared to PRP group ($p>0.05$) (Table 3).

3. Histologic evaluation

In all specimens, the defects were completely closed by the PTFE membrane and all group showed an increase in bone formation over time(Figure 6,7,8). The perforated areas were filled with loose fibrous tissue in control group and filled with dense fibrous tissue in PRP group(Figure. 6 A,B). There was active osteoblastic activity and immature bone formation at the border of the defect in control and PRP group, it was similar histologically between control and PRP group. A slightly increase osteoblastic and osteoid layers was seen when PRP group was compared with control(Figure. 6,7,8 A,B). A slightly increase in osteoblastic and osteoid layers was seen for Bio-Oss® group compared with control and PRP group(Figure 6,7,8 A,B,C). There were osteoblastic and osteoid layers at the border of the defect and around grafted bone particles in Bio-Oss® group and Bio-Oss® with PRP group at 1 week(Figure 6 C,D), more osteoblastic and osteoid layers of Bio-Oss® with PRP group were seen than that of Bio-Oss® group. There was newly formed bone at the border of the defect and around grafted bone particles in Bio-Oss® group and Bio-Oss® with PRP group at 2, 4 weeks(Figure 7,8 C,D). The grafted bone particles have been incorporated in mature new bone and

Table 2. Platelet Counts (platelet count/㎣): 402% increase

	Blood	PPP	PRP
A	167,000	10,000	664,000
B	148,000	10,000	425,000
C	174,000	45,000	723,000
D	112,000	60,000	752,000
E	159,000	50,000	597,000
F	143,000	28,000	475,000
평균	150,500	34,000	606,000

Blood : whole blood, PPP : platelet-poor plasma, PRP : platelet-rich plasma

Table 3. Amount of bone fill determined radiographically over the 4 weeks.

	1 week	2 week	4 week
control	0.02 ± 0.01	0.04 ± 0.02	0.21 ± 0.05
PRP	0.04 ± 0.01	0.13 ± 0.02	0.24 ± 0.04
Bio-Oss®	0.65 ± 0.06*	0.94 ± 0.15*	1.16 ± 0.02*
Bio-Oss® + PRP	0.76 ± 0.17*	1.03 ± 0.08*	1.20 ± 0.06*

mean ± SD (gram/square inch) analyzed by a ImageJ 1.31v software

statistical analysis : one-way ANOVA with fisher's Tukey test ; P<0.05

* : Significantly different from corresponding control (P<0.05)

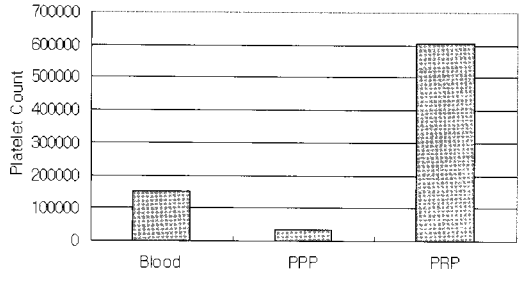

Figure 4. Average platelet counts

Blood : whole blood, PPP : platelet-poor plasma, PRP : platelet-rich plasma

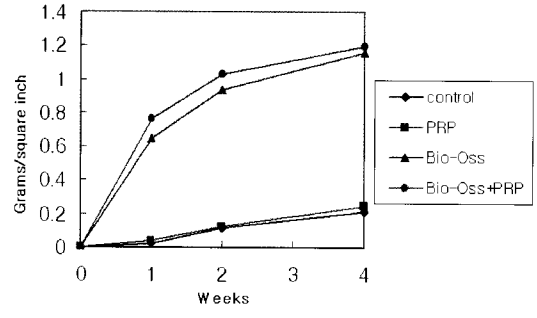

Figure 5. Amount of bone fill determined radiographically over the 4 weeks.

was resorbed during the remodeling process in Bio-Oss® with PRP group at 4 weeks(Figure 8 D). New bone formation was increased in Bio-Oss® with PRP compared those of Bio-Oss® alone.

All group showed an increase in bone formation at 4 weeks as compared with 1,2 weeks (Figure 6,7,8). There was no difference at newly formed bone when control group was compared with PRP group (Figure 6,7,8 A,B) and Bio-Oss® group was compared with Bio-Oss® with PRP group (Figure 6,7,8 C,D). The individual particles of the bovine bone material were clearly identifiable and they were found to be surrounded by varying amounts of newly formed bone without being encapsulated by loose fibrous connective tissue in Bio-Oss® and Bio-Oss® with PRP group at all time.

IV. Discussion

In the scope of bone regeneration, the augmentation of bone defects using autogenous bone to be the gold standard. But, autogenous bone is not always available in sufficient volume for grafting. The ideal grafting material remains the subject of continued research. Researchers strive continuously to improve upon current bone grafting techniques and provide faster and denser regeneration. Growth factors are a realistic way to improve both soft tissue and bony wound healing. Platelets contain angiogenic, mitogenic, and vascular growth factors in

their granules[28]. TGF-β, PDGF, and VEGF are known to ve produced by platelets and released during degranulation. TGF-β has been shown to stimulate proliferation and collagen synthesis by osteoblasts and osteoblast precursors[25]. It also may act as a chemotactic agent in recruiting pre-osteoblasts to the site of bone injury. PDGF also stimulates mitogenesis of osteoblastic precursors[29,30]. Although there are numerous cytokines and growth factors that play a role in the specific temporal sequence that occurs during bone graft healing, TGF-β and PDGF most likely contribute to the early influx of cells and stimulation of proliferation. The biologic of autogenous and recombinant growth factors such as TGF-β and PDGF and underlying mechanisms were investigated in numerous studies[31,32]. These factors belong to a class of biologic mediators with an important stimulatory and regulatory function on cellular processes such as mitogenesis, differentiation, and chemotaxis, as well as angioneogenesis during bone and soft tissue healing. Studies on application of single or combination growth factors using PDGF or PDGF/IGF-1 have been shown to enhance the early cascade of tissue repair processes both in vitro[33,34]and in vivo[35,36,37].

Marx and associates[25] attributed the osteoregenerative effect of PRP to an increased release of PDGF and TGF-β and the resultant enhanced stimulation of angiogenesis, mitogenesis of marrow stem cells and preosteoblasts, and their activation and differentiation into mature osteoblasts. In vitro studies showed that the combination of certain cytokines and growth factors increased osteoblast proliferation and differentiation[38]. Also Kawase and associates[39] suggested that growth factor might have a potential for enhancing collagen synthesis in periodontal ligament and osteoblastic cells. It is therefore a reasonable hypothesis that increasing the concentration of platelets in a bone defects in a bony defect may lead to improved and faster healing. Recent reports have suggested that more rapid epithelialization, more dense and mature bone with better organized trabeculae, and greater bone regeneration take place when PRP is added to bone autografts and allografts[25,40,41]. Most of these reports also suggest that PRP improves the handling properties of the grafts material with which it is combined, facilitating graft placement and stability.

Marx and coworkers[25] performed the first and most compelling study available on the use of PRP in combination with bone grafts. The authors claimed that the bone grafts combined with PRP showed a maturity index more than twice and slightly less than twice the actual maturity at 2 and 4 months, respectively.

Anitua[40] reported the results of the use of PRP in a series of patients who underwents tooth extraction because of root fracture or periodontitis. The sites treated with PRP demonstrated more mature bone, with better organized trabeculae and greater bone regeneration. Also, the epithelialization was described based on subjective observations as very good to excellent. PRP is thought to accelerate soft tissue healing by promoting a more rapid revascularization and reepithelialization of flaps and cell proliferation. However, because of the limited ability to extrapolate the data to human conditions, further investigations of PRP in combination with xenograft materials such as anorganic bovine bone is obviously necessary.

Bio-Oss® is natural bovine bone that is completely deproteinized to prevent a potential immune response[42,43]. Electron microscopic evaluation shows that this material has a structural configuration similar to human bone. Its compressive strength and modulus of elasticity are also similar to the values for human bone[44].

Khalid[45] reported that the bovine bone materials posseessed the best potential of osteoconductive grafting material, followed by the biograss crystals and the hydroxyapatite particles respectively. In this study, the bovine bone materials showed significant increase in newly formed bone when compared to the no graft as a control. Also, there was more newly formed bone area in the bovine bone group than in the control group at 1, 2, 4 weeks.

In this study, radiographic assessment did not show any significant difference between Bio-Oss® group and Bio-Oss® with PRP group. It showed a significant increase in bone density when Bio-Oss® and Bio-Oss® with PRP were grafted, compared to ungrafted control and PRP group, at nearly every evaluation point. The clinical significance of these data is difficult to determine because any radiopaque bone grafting material will look more dense on a radiograph. However, this is not consistent with previous studies, which showed significantly greater bone density at 1 and 2 months when evaluating digitized radiographs and computed tomography scans when PRP was added to Bio-Oss ® in rabbit cranial defects[17]. Also, Marx and associates[25] showed a 1.62- to 2.16-fold increase in radiologic bone density at 6 and 2 months, respectively, when PRP was added to autogenous bone, as evaluated with panoramic radiographs. The previously report, however, studied human mandibular continuity defects.

In the present study Bio-Oss® group and Bio-Oss ® with PRP group showed a significant increase in newly formed bone when compared to control and PRP group at all time. Also, adding PRP to Bio-Oss® resulted in a significant increase in bone area at all time periods compared with Bio-Oss® alone. Suba and associates[46] reported the results of the use of PRR in extraction site of beagle dog. The combined use with beta-tircalcium phosphate (Cerasorb®) and PRP results in more intense bone regeneration, especially in early phase.

Aghloo[47] showed a histomorphometric increase in bone formation with the addition of PRP to Bio-Oss® in non-critical sized defects in the rabbit cranium. These results conflict with those of Wiltfang and associates[48]. They reported that the bone regenaration in critical-size defects of pig's frontal region. PRP did not add additional benefit when xenogenic bone substitute were used. however, a significant effect on bone regeneration was found in the autogenous group initially when PRP in added. Also, Furst[49] reported that minipig sinus grafted core samples were evaluated histological after grafting with bovine hydroxyapatite alone and bovine hydroxyapatite with PRP. The authors found no histologic benefit by the addition of PRP.

These inconsistent results may be due to the different experimental systems and different animal employed in their experiments. Therefore the bioactive effects of PRP on bone wound healing and mineralized tissue formation depend on the local osseous environment where PRP has been applied. Also, the majority of the bony regeneration took place within the first month of healing. Significant differences might have been seen in early wound healing between bone alone and bone with PRP, had samples been evaluated before 1 month.

In conclusion, this study has clearly demonstrated that the addition of PRP to Bio-Oss® in the rabbit cranial defect model was shown to be potentially beneficial at early bone healing. Also, Deproteinized bovine bone materials had the good osteoconductive properties and served as a space maintainer successfully. However, this study was designed to evaluated non-critical sized cranial defects. Further studies need to evaluate the potential benefits of PRP in healing critical sized defects.

V. Conclusion

Platelets are very important in the wound healing process. They quickly arrive at the wound site and begin the coagulation process. They release multiple wonud healing growth factors and cytokines, including platelet-derived growth factor (PDGF), transforming growth factor-β(TGF-β), insulin-like growth factor-1, vascular endothelial cell growth factor (VEGF), platelet-derived epidermal growth factor, platelet factor 4. These growth factors are thought to contribute to initial bone regeneration and increased vascularity, vital features of a healing bone graft. This study is to evaluate the effect of PRP on the early wound healing of rabbit cranial defects.

Fifteen New Zealand white male rabbits between 2.8 and 4 kg were included in this randomized, blinded, prospective study. By means of a trephine bur (external diameter : 8mm), four standardized 'through-and-through' bone defect were created with copious irrigation. The four cranial defects were randomly grafted with Bio-Oss®, Bio-Oss® mixed with PRP, PRP alone, and no graft as a control. The Four defects were covered with nonresorbable PTFE membrane (Tefgen®, Lifecore Biomedical, Inc., U.S.A.). The wound was closed with resorbable suture materials. Rabbits were killed using phentobarbital, 100 mg/kg intravenously at 1, 2, and 4 weeks. Radiographs were taken of the rabbit cranium in its entirety before histologic sections were performed. A aluminum step-wedge was used in each radiograph for camparison. Specimens were treated with hydrochloric acid decalcifying solution (Fisher Scientific, Tustin, CA) and sectioned by bisecting the 8-mm diameter defects. The histologic specimens were prepared in the usual fashion with H&E staining at 6 ㎛ in thickness. Also, six specimens were sampled analysed for platelet count. The following results were obtained through the in *vivo* study.

1. In platelet count test, PRP group is 4 times higher platelet concentration than normal.
2. In radiographic evaluation, Bio-Oss® group and Bio-Oss® with PRP group showed a significant increase in radiographic bone density when compared to the control and PRP group at all 3 time points ($p<0.01$). However, significant increase was not seen at all time when control group was compared with PRP group ($P>0.05$). There was also no significant difference between Bio-Oss® and Bio-Oss® with PRP group at 1, 2, and 4weeks ($P>0.05$).
3. In histologic evaluation, all grafting materials showed an increase in bone formation over a time. Bio-Oss® and Bio-Oss® with PRP group showed a increase in newly formed bone when compared to control and PRP group at 1, 2 and 4 weeks. Also, a increase in new bone formation was seen when Bio-Oss® with PRP was compared with Bio-Oss® alone.

The results has suggested that the addition of PRP to Bio-Oss® in the rabbit cranial defects model was shown to be potentially beneficial at early bone healing. PRP might positively influence the early bone wound healing.

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사진부도 설명

Figure 6 (A) A light micrograph of control at 1 week postoperatively: The perforated areas were filled with loose fibrous tissue. There was osteoblastic and osteoid layers from cortical bone margin.

(B) A light micrograph of PRP group at 1 week postoperatively : The perforated areas were filled with dense fibrous tissue. There was osteoblastic and osteoid layers from cortical bone margin.

(C) A light micrograph of Bio-Oss group at 1 week postoperatively : There were osteoblastic and osteoid layers at the border of the defect and around deproteinized bovine bone material particles.

(D) A light micrograph of Bio-Oss with PRP group at 1 week postoperatively: There was formation of new bone from cortical bone margin. Osteoprogenitor cells and preosteoblasts were seen on the periphery of the graft materials.

Figure 7 (A) A light micrograph of control at 2 week postoperatively : The perforated areas were filled with dense fibrous tissue. There was formation of new bone from cortical bone margin.

(B) A light micrograph of PRP group at 2 week postoperatively : The perforated areas were filled with dense fibrous tissue. There was formation of new bone from cortical bone margin.

(C) A light micrograph of Bio-Oss group at 2 week postoperatively : There was formation of new bone from cortical bone margin. Osteoprogenitor cells and preosteoblasts were seen on the periphery of the graft materials.

(D) A light micrograph of Bio-Oss with PRP group at 2 week postoperatively : There was formation of new bone from cortical bone margin. The graft materials have been incorporated into the newly formed bone matrix.

Figure 8 (A) A light micrograph of control at 4 week postoperatively : The perforated areas were filled with dense fibrous tissue. There was formation of new bone from cortical bone margin.

(B) A light micrograph of PRP group at 4 week postoperatively : The perforated areas were filled with dense fibrous tissue. There was formation of new bone from cortical bone margin.

(C) A light micrograph of Bio-Oss group at 4 week postoperatively : There was formation of new bone from cortical bone margin. The graft materials have been incorporated into the newly formed bone matrix.

(D) A light micrograph of Bio-Oss with PRP group at 4 week postoperatively : There was formation of new bone from cortical bone margin. The graft materials have been incorporated in mature new bone and was resorbed during the remodeling process.

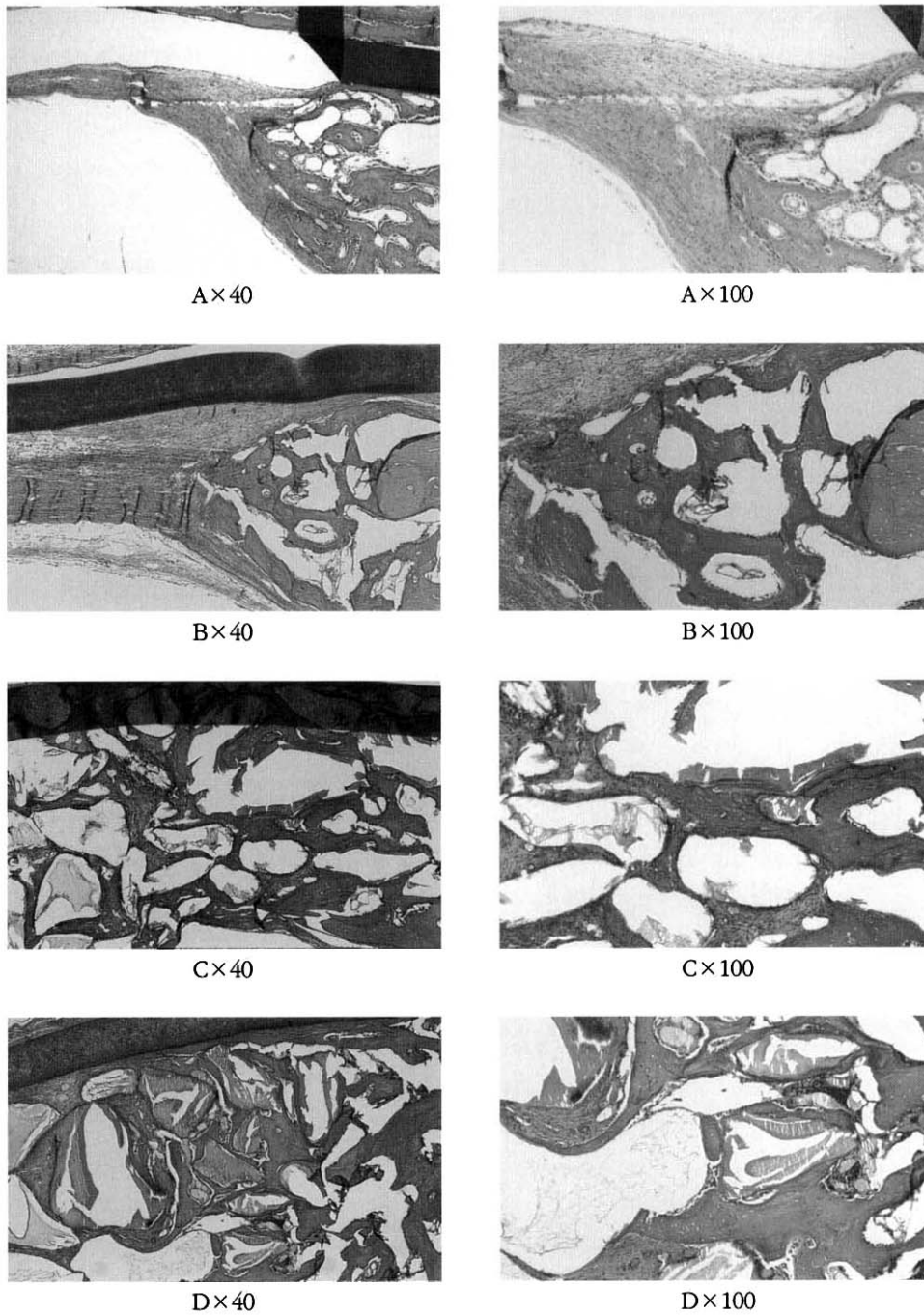
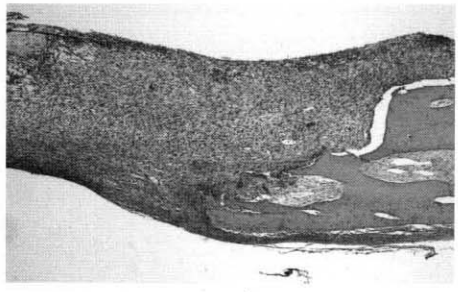


Figure 6. Light micrographs at 1 week postoperatively; control (A), PRP (B), Bio-Oss® (C), and Bio-Oss® with PRP (D). (H&E×40, ×100)



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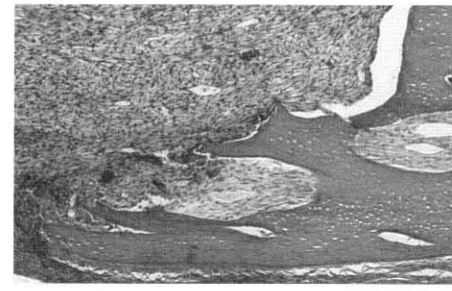

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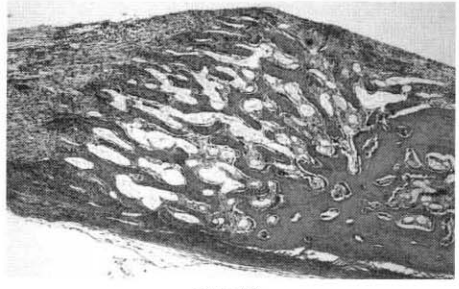

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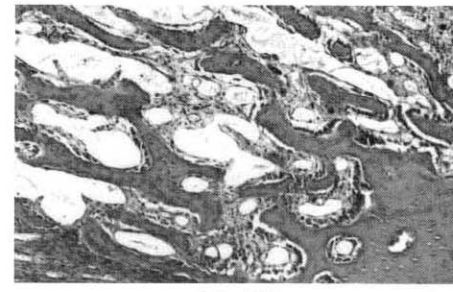

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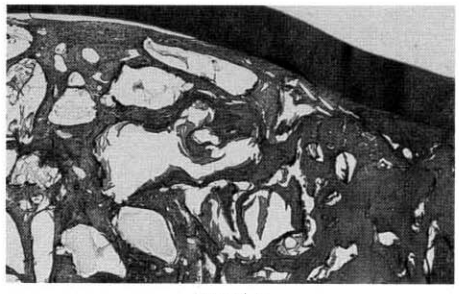

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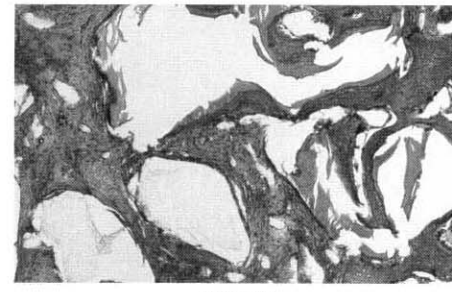

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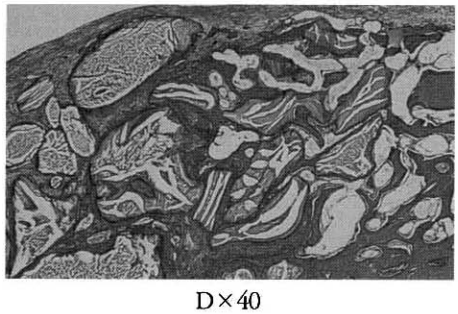
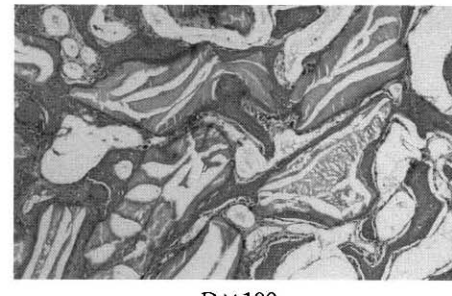


D×40



D×100

Figure 7. Light micrographs at 2 week postoperatively; control (A), PRP (B), Bio-Oss® (C), and Bio-Oss® with PRP (D). (H&E×40, ×100)

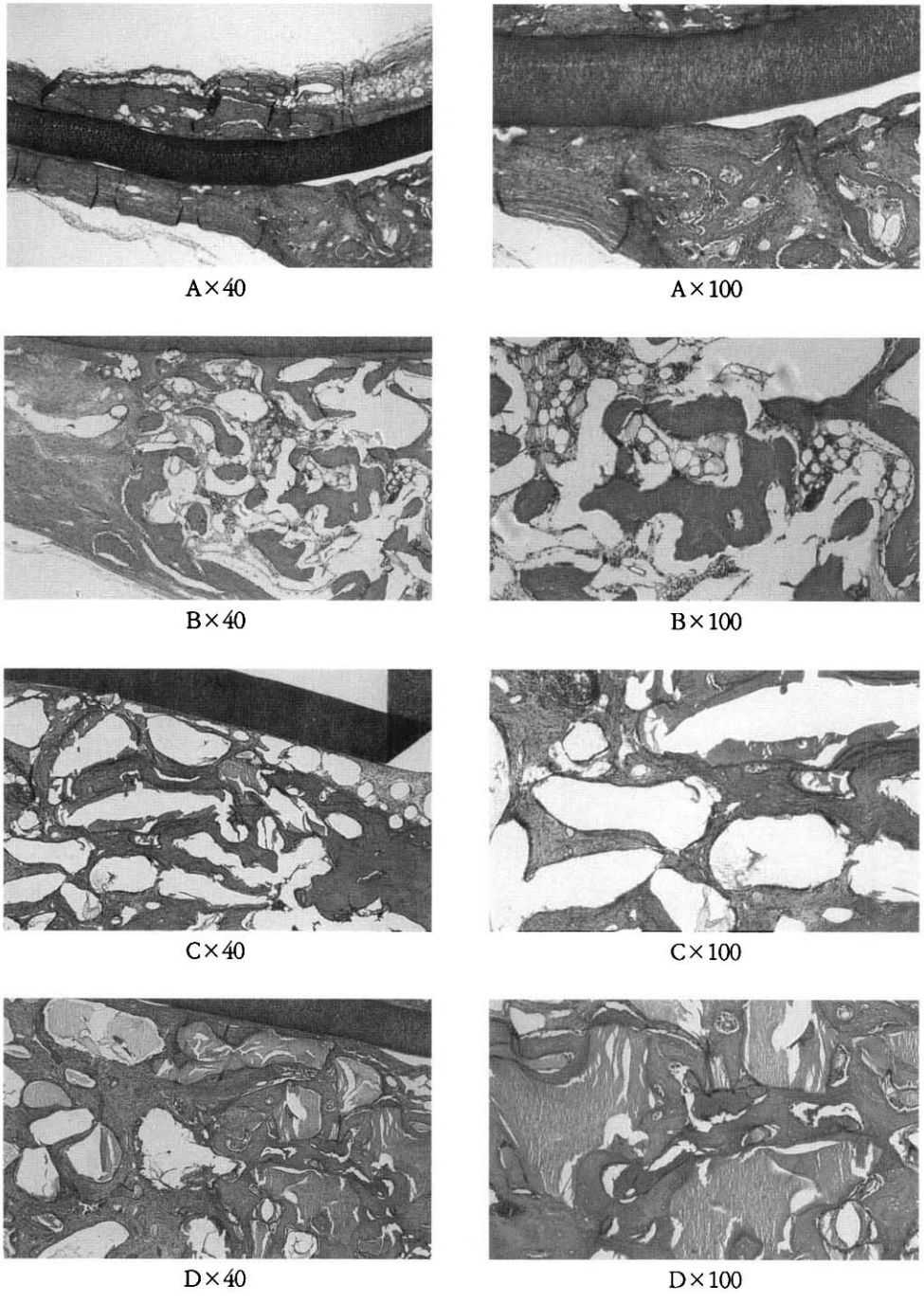


Figure 8. Light micrographs at 4 week postoperatively; control (A), PRP (B), Bio-Oss® (C), and Bio-Oss® with PRP (D). (H&E×40, ×100)

-국문초록-

혈소판 농축 혈장과 혼합된 이종골 이식재(Bio-Oss®)가 가토 두개골 결손부 초기 치유에 미치는 영향

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혈소판 농축 혈장은 구강과 안면부 재건수술에 새로이 사용되는 유용한 첨가물이다. 혈소판은 상처 치유과정에서 매우 중요하며, 혈소판은 상처부위에 빠르게 도달하여 응고를 형성한다. 그리고 다양한 성장인자를 분비한다. 이러한 성장인자는 골의 형성과 혈관의 증가, 골 이식재의 치유에 관여하는 것으로 생각된다. 본 연구의 목적은 실험 동물을 통하여 혈소판 농축 혈장에 함유된 혈소판의 정량화를 통한 성장인자 함유량을 추정하고, 방사선학적, 조직학적 평가를 통해 혈소판 농축 혈장이 초기의 골형성에 미치는 영향에 대한 평가를 하는데 있다.

15마리의 가토 두개골에 6 mm trephine bur(외경 8 mm)를 이용하여 경뇌막의 손상을 주지 않도록 하면서 4개의 결손부를 형성하였다. 각각의 두개골 결손부는 Bio-Oss®만 이식한 군, PRP만 이식한 군, PRP와 Bio-Oss®를 혼합하여 이식한 군, 그리고 아무것도 이식하지 않은 군을 대조군으로 설정하였다. 각각의 재료를 이식한 후 비흡수성 차폐막(Tefgen®)을 위치시키고 흡수성 봉합사로 일차봉합을 시행하였다. 각 군 당 술 후 1, 2, 4주의 치유기간을 설정하였다. 동물을 희생시키고 두개골을 절제하였다. 먼저 방사선학적인 골 밀도 측정을 시행하고, 조직학적 평가를 위해 통법에 따라 조직 표본을 제작한 후 광학현미경으로 관찰하였다. 또한 가토 귀 변연정맥에서 채취한 10 *ml*의 혈액을 원심분리하여 혈소판 함유량을 평가하여 다음과 같은 결과를 얻었다.

1. 혈소판 농축 혈장은 일반 혈액에 비해 약 4.02배 많은 수의 혈소판이 함유되어 있었다.
2. 방사선적인 평가에서 1, 2, 4주 사이에 대조군과 비교하여 Bio-Oss®에 PRP를 이식한 군에서 골의 밀도는 큰 차이를 보이고 있다($p<0.01$). 하지만, 동일한 시기에 PRP만 이식한 군과 대조군의 차이는 발견할 수 없었으며($p>0.05$), Bio-Oss®만 이식한 군과 Bio-Oss®에 PRP를 이식한 군의 차이 또한 발견할 수 없었다($p>0.05$).
3. 조직학적 평가에서 모든 이식재는 시간이 경과할수록 골 형성이 증가함을 알 수 있었다. 대조군에 비해 PRP만 이식한 군에서 더 두꺼운 섬유성 결합을 보이고 있다. 대조군과 PRP만 이식한 군과 비교해 Bio-Oss®와 Bio-Oss®에 PRP를 혼합 이식한 군에서 골의 형성이 더 진행됨을 알 수 있었다. Bio-Oss® 에 PRP를 혼

합 이식한 군이 Bio-Oss®만 이식한 군에서보다 더 많은 신생골 형성을 관찰할 수 있다.

이상의 결과에서 가토의 두개골 결손부에 Bio-Oss®에 PRP를 혼합 이식하였을 경우 결손부의 초기 골 형성을 촉진 할 수 있음을 시사하였다.

주요어 : 혈소판 농축 혈장, Bio-Oss® ,PRP